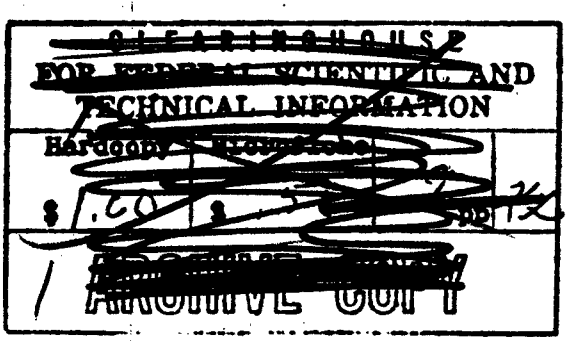
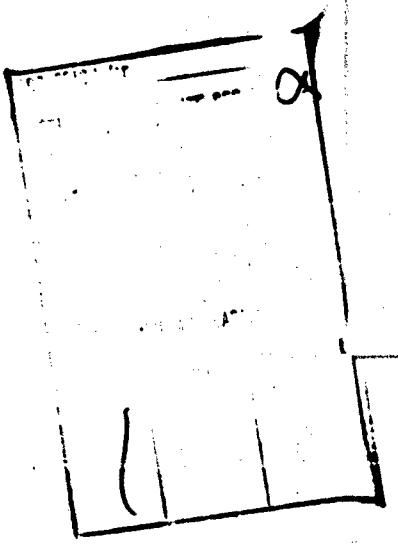
ALSO TT

STANDARDIZATION OF CONDITIONS FOR SETTING UP THE HEMAGGLUTINATION INHIBITION REACTION WITH THE NONINFECTIVE ANTIG OF THE TICK-BORNE ENCEPHALITIS VIRUS

Translation No. 1606



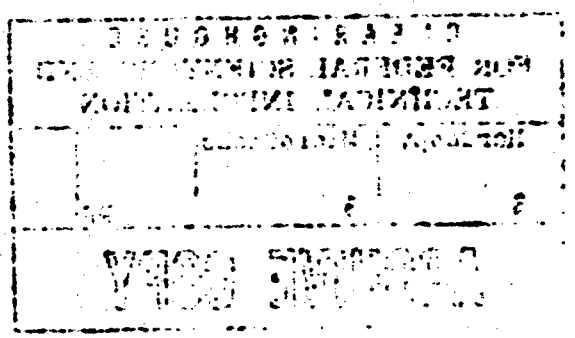
U. S. ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND



DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Va.



Technical Library Branch
Technical Information Division

STANDARDIZATION OF CONDITIONS FOR SETTING UP THE HEMAGGLUTINATION INHIBITION REACTION WITH THE NONINFECTIVE ANTIGEN OF THE TICK-BORNE ENCEPHALITIS VIRUS

[Following is the translation of an article by A. I. Rezepova, Moscow Scientific-Research Institute of Viral Preparations, published in the Russian-language periodical Voprosy virusologii (Problems of Virology) 8(2), 1963, pages 180-184. It was submitted on 28 May 1962. Translation performed by Sp/7 Charles T. Ostertag Jr.]

The extensive application of the hemagglutination inhibibition reaction (RTGA) for the purpose of investigating the immunological condition of the population and animals in foci of disease requires the development of a common standard method in order that the different investigators obtain comparable results.

Up until the present time there is no common arrangement for setting up the RTGA: There are variations in temperature conditions, the concentration of erythrocytes, the contact time between the antigen and the serum and other factors which directly influence the results of the reaction [1,2].

In the present work we have undertaken the mission to study the influence of various factors on the results of the hemagglutination reaction (RGA) and the RTGA and to establish the optimum conditions for setting up these reactions. The tests were conducted with the noninfectious antigen of the tick-borne encephalitis virus, prepared by a method which we had proposed earlier [3] and which is widely used at the present time in the USSR. The necessity of such a type of investigations is also connected with the fact that in earlier published works infectious antigens were used which were prepared by various methods.

Methods and Materials

The basis of the method for setting up the RGA and the RTGA was the arrangement proposed by Porterfield [4] and used by many investigators.

The dilutions of antigen and sera were prepared in borate buffer solution with pH of 9.0. The reaction was set up in a volume of 0.8 ml (0.4 ml of antigen and 0.4 ml of erythrocytes in the RGA and 0.2 ml of serum, 0.2 ml of antigen and 0.4 ml of erythrocytes in the RTGA). All the solutions were preliminarily cooled. In the event of the formation of a layer of erythrocytes uniformly covering the bottom of the test tube in the form of an umbrella, the reactions were considered positive, and with the settling of the erythrocytes in the form of a disk -- negative. As the titer of the antigen we used the

last dilution in which it was possible to note the typical agglutination of erythrocytes.

We considered it necessary to study the feasibility of using in the RGA and the RTGA the erythrocytes of man and various species of animals: Calves, sheep, monkeys, rabbits, guinea pigs, hamsters, white mice, roosters and geese. To avoid coagulation the blood was mixed with Alsever's solution (1 volume of solution for 4 volumes of blood) and washed three times with a buffered physiological solution. The washed erythrocytes were stored at 4°.

We studied the influence of the concentration of erythrocytes, the amounts of the working dose used in the test, temperature conditions and the duration of contact between the antigen and serum on the results of the RTGA and the RGA.

Results

The results of the RGA with the erythrocytes of man, sheep, geese and roosters at various pH values of the buffer solutions are presented in table 1. High hemagglutination titers were obtained with the erythrocytes of geese (1:1280), roosters (1:640) and sheep (1:320).

The tests showed that the phenomenon of hemagglutination with the erythrocytes of man and geese is not produced constantly. In comparison with other erythrocytes, the erythrocytes of geese are agglutinated in a wider pH range. The erythrocytes of other animals either did not produce the phenomenon of hemagglutination with the antigen of the tick-borne encephalitis virus, or else spontaneous hemagglutination was observed. The erythrocytes of geese and sheep are the most suitable for utilization in the reaction, since they produce the high and regularly reproducible results over a period of 10 - 14 days from the time they are obtained.

In the following tests only the goose erythrocytes were used.

The results of a comparative study of the RGA and the RTGA, obtained with various concentrations of goose erythrocytes at a constant dose of antigen, are presented in table 2. It can be seen from the table that a concentration of 0.1% of erythrocytes produces results which are difficult to consider. An increase of the concentration by 7 times (from 0.2 to 1.4%) is accompanied by a lowering of the titer of the antigen by 8 - 16 times. A change in the concentration of erythrocytes influences the results of the RTGA to a lesser degree

The time during which the agglutination of erythrocytes takes place is found to be directly dependent on the concentration of the latter. The higher concentrations (0.5 - 1.4%) make it possible to obtain clearer and more rapidly calculable results, which remain unchanged for several hours. A concentration of 0.2% may be successfully used in conjunction with the minimum amounts of antigen.

For determining the optimum concentration of erythrocytes which should be used in the RTGA, we conducted tests with various concentrations. In all the tests we used 8 working doses of antigen which were calculated according to the results of the RGA for each individual concentration of erythrocytes.

As is seen from table 3, the same serum titers were obtained under these conditions regardless of the concentration of erythrocytes. Consequently it is possible to use various concentrations of erythrocytes, however it is necessary to use the same concentration of them for determining the working dose of antigen and the titer of the serum.

One of the important steps in setting up the RTGA is the correct selection of the dose of antigen. As is seen from table 4, an increase in the number of working doses from 8 to 32 leads to a lowering of the serum titer by 2 - 4 times.

The data presented in table 5 shows that the optimum temperature for the agglutination of erythrocytes is a temperature of 4°. Under these conditions the highest titers of antigen are obtained. An increase of temperature from 4 to 37° is accompanied by a lowering of the titer of the antigen by 4 - 8 times and an increase in the titer of the sera by 2 - 4 times.

A significant influence on the results of the RTGA is exerted by the duration of contact of the antigen with the serum, and also the temperature conditions during the time of contact. The formation of the neutral complex of antigen--antibody takes place both at 4° and at 37° (table 6). The maximum periods, guaranteeing the maximum fullness of binding the antigen with the antibody, are 5 hours at 37° and 24 hours at 4°. A further increase of the periods of contact leads to a disruption of the hemagglutinating properties of the antigen.

In parallel tests with immune rabbit serum we made a comparison of results obtained with the infectious and inactivated antigen of the tick-borne encephalitis virus. The necessity of such a type of investigations is connected with the fact that during the inactivation of the antigen chemical substances are introduced into it which may influence the phenomenon of hemagglutination.

However the results of the test showed that based on its hemagglutinating properties the inactivated antigen is identical to the infectious one.

In connection with what has been presented above we consider it necessary to adhere to a specific standard method of setting up the RGA and the RTGA in order to make it possible to obtain comparable results in various laboratories.

1. In the RTGA only 8 working doses of antigen should be used.

2. The duration of contact of the antigen with the serum at 37° should be 5 hours, and at 4° - 24 hours.

3. Both goose and sheep erythrocytes can be used in the reactions. There concentration should be the same in the RGA and the RTGA and comprises 0.5 - 1.4%.

4. The settling of erythrocytes in the RGA should be done or carried out at 4°, since an increase of temperature in this case will lead to a very high number of working doses in the RTGA.

Literature

a. Ilyenke, V. I., Voprosy virusologii, 1961, No 4, page 495.
b. Levkovich, Ye. N., Izotov, V. K., Ibid, page 428.
c. Semenov, B. F., Rezepova, A. I., Ibid, page 432.
d. Porterfield, J. S., Nature, 1957, v 180, page 1201.

Table 1

Agglutination of various types of erythrocytes in the presence of noninfectious antigen at various pH values.

Erythrocytes (concentration 0.5%)	pH								Spontaneous hemagglutination
	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	
Geese	1:320	1:320	1:640	1:1280	1:1280	1:1280	1:40	1:20	None
Sheep	1:20	1:40	1:80	1:160	1:320	1:160	1:20	0	"
Roosters	1:320	1:320	1:640	1:640	1:320	1:160	0	0	In individual specimens
Man	—	1:80	1:160	1:160	1:160	1:40	0	0	None

Table 2

Influence of the concentration of goose erythrocytes on the results of the RGA and the RTGA.

Concentration of erythrocytes (in %)	Titer of antigen in RGA			Titer of serum in RTGA			Time of appearance of agglutination (min.)
	No. of test			No. of test			
	1	2	3	1	2	3	
0.1			Results not clear				
0.2	1:2560	1:1280	1:2560	1:320	1:160	1:160	45-60
0.3	1:1280	1:640	-	1:320	1:160	-	
0.4	1:1280	1:640	1:1280	1:320	1:160	-	
0.5	1:640	1:640	1:1280	1:320	1:160	1:160	35
0.6	1:320	1:320	1:640	1:320	1:320	1:160	
0.7	1:320	1:160	1:320	1:320	1:320	1:160	30
1	1:320	1:160	1:160	1:320	1:320	1:160	20
1.2	1:320	1:160	1:160	-	1:640	-	15
1.4	1:320	1:160	1:160	1:160	1:640	1:320	

Table 3

Dependency of the results of the RTGA on the concentration of goose erythrocytes.

Concentration of erythrocytes (in %)	Titer of the antigen in the RGA	Dilution of antigen corresponding to 8 working doses	Titer of serum in the RTGA			
			No. of test			
			1	2	3	4
0.2	1:320	1:40	1:80	1:160	1:40	1:320
0.5	1:160	1:20	1:80	1:160	1:40	1:320
1.4	1:40	1:5	1:80	1:160	1:40	1:320

Table 4

Influence of the number of working doses of antigen on the results of the RTGA.

Number of working doses of antigen	Titer of serum					
	No. of test					
	1	2	3	4	5	6
8	1:1280	1:1280	1:1280	1:1280	1:1280	1:640
16	1:640	1:1280	1:640	1:640	1:640	1:320
32	1:320	1:640	1:320	1:320	1:320	1:160

Table 5

Influence of temperature on the results of the RGA and the RTGA.

Temperature	Titer of antigen in the RGA			Titer of serum in the RTGA		
	No. of test					
	1	2	3	1	2	3
37°	1:160	1:320	1:160	1:640	1:2560	1:2560
18°	1:320	1:160	1:320	1:320	1:1280	1:1280
14°	1:640	1:320	1:640	1:320	1:1280	1:640
4°	1:640	1:1280	1:320	1:320	1:1280	1:640

Table 6

Influence of the duration of contact of the antigen with the serum and the temperature conditions at the moment of contact on the results of the RTGA.

Number of serum	Temperature	Period of contact (in hours)					
		0	1	2	3	5	24
1	4° 37°	1:20	1:40 1:80	1:40 1:80	1:80 1:320	1:80 1:640	1:640 o
2	4° 37°	1:40	1:80 1:160	1:80 1:160	1:80 1:320	1:160 1:640	1:1280 0
3	4° 37°	1:20	1:80 1:80	1:80 1:80	1:160 1:320	1:160 1:640	1:640 0